Published in final edited form as:

J Neurosci Methods. 2015 March 15; 0: 106–111. doi:10.1016/j.jneumeth.2015.01.014.

Impact of immersion oils and mounting media on the confocal imaging of dendritic spines

Brittni M. Peterson^{a,b}, Paul G. Mermelstein^{a,b}, and Robert L. Meisel^{a,b,*}

^aGraduate Program in Neuroscience, 6-145 Jackson Hall, 321 Church Street SE, University of Minnesota, Minneapolis, MN 55455, USA

^bDepartment of Neuroscience, 6-145 Jackson Hall, 321 Church Street SE, University of Minnesota, Minneapolis, MN 55455, USA

Abstract

Background—Structural plasticity, such as changes in dendritic spine morphology and density, reflect changes in synaptic connectivity and circuitry. Procedural variables used in different methods for labeling dendritic spines have been quantitatively evaluated for their impact on the ability to resolve individual spines in confocal microscopic analyses. In contrast, there have been discussions, though no quantitative analyses, of the potential effects of choosing specific mounting media and immersion oils on dendritic spine resolution.

New Method—Here we provide quantitative data measuring the impact of these variables on resolving dendritic spines in 3D confocal analyses. Medium spiny neurons from the rat striatum and nucleus accumbens are used as examples.

Results—Both choice of mounting media and immersion oil affected the visualization of dendritic spines, with choosing the appropriate immersion oil as being more imperative. These biologic data are supported by quantitative measures of the 3D diffraction pattern (i.e. point spread function) of a point source of light under the same mounting medium and immersion oil combinations.

Comparison with Existing Method—Although not a new method, this manuscript provides quantitative data demonstrating that different mounting media and immersion oils can impact the ability to resolve dendritic spines. These findings highlight the importance of reporting which mounting medium and immersion oil are used in preparations for confocal analyses, especially when comparing published results from different laboratories.

© 2015 Elsevier B.V. All rights reserved.

*Corresponding author meisel@umn.edu Office: 1-612-625-6796 Fax: 1-612-626-5009.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Ethical Standards This manuscript does not contain clinical studies or patient data.

Conflict of Interest The authors declare that they have no conflict of interest.

Conclusion—Collectively, these data suggest that choosing the appropriate immersion oil and mounting media is critical for obtaining the best resolution, and consequently more accurate measures of dendritic spine densities.

Keywords

confocal microscopy; dendritic spines; synaptic plasticity; refractive index

1. Introduction

Dendritic spines are small structures that protrude from the dendritic shaft where they receive the majority of glutamatergic inputs for a neuron (Alvarez and Sabatini 2007). They are highly dynamic structures (Bhatt et al. 2009), for which alterations in structure or density imply changes in neuroanatomical circuitry, neuronal function and consequently behavior. Given these properties, confocal-based dendritic spine analyses are common in the neuroscience literature, despite the challenges of resolving small spine structures with varying geometries that are located in close proximity to the dendritic shaft and other spines. Following confocal laser scanning microscopy, optical sections through dendritic segments are reconstructed into representative 3D images. However, light scattering can obscure distinct structures, and limit the ability to distinguish between individual dendritic spines.

Recently, there has been greater attention towards systematically testing and quantitatively evaluating procedural variables used in preparing tissue for confocal 3D measurements of dendritic spines (Dumitriu et al. 2011; Staffend and Meisel 2011b). There has been additional focus on the imaging parameters necessary to achieve optimal resolution of dendritic spines (Dumitriu et al., 2011). In addition to optimizing imaging parameters, these authors suggested that experimenter-controlled variables such as proper selection of mounting medium and immersion oil may have important consequences for resolution (Dumitriu et al. 2011). Surprisingly, experiments that empirically test the impact of choice of immersion oil and mounting medium on resolution of small biological structures, such as dendritic spines are limited.

Throughout our experimentation over the last several years, we have examined how mounting media and immersion oils of different refractive indices affect the resolution of individual dendritic spines and consequently dendritic spine densities of dialkylcarbocyanine (DiI)-labeled striatal medium spiny neurons (MSNs). We have examined two separate choices for mounting media: glycerin, a versatile non-hardening compound widely used in fluorescent microscopy and Fluorglo, a recently developed hardening compound compatible with fluorescence. Likewise, we examined two distinct immersion oils: Type FF, with a refractive index that closely matches the mounting media versus Type LDF, with a refractive index that matches the glass through which the sample is visualized.

Here we report that the choice of mounting medium has a small effect on optical resolution, and dependent on brain region, may influence the ability to resolve individual dendritic spines. In contrast, immersion oil has a larger impact on 3D resolution. Specifically, matching the immersion oil refraction index to the glass reliably enhanced the ability to visualize dendritic spines.

2. Materials and Methods

2.1 Point Spread Function Analyses of Microspheres

2.1.1 Preparation of FluoSpheres—Instructions for preparing sub-resolution fluorescent microspheres are detailed in (Cole et al. 2011). Briefly, number 1.5 coverglass (Zeiss) and slides (Brain Research Laboratories; Newton, MA) were washed in 70% ethanol and flamed to dry, after which 100 nm red fluorescent microspheres (FluoSpheres carboxylate-modified microspheres 580/605; Invitrogen, Molecular Probes; Eugene, OR) diluted at 1:25,000 in dH₂O were sonicated for 20 min, vortexed and applied in 20 μ l aliquots to the coverglass. FluoSpheres were allowed to dry onto the coverglass (~2hr) and mounted to slides using 5% n-propyl-gallate in glycerin (w/v) or Fluorglo mounting medium for lipophilic dyes (Spectra Services, Ontario, NY) with a 120 μ m secure seal spacer (Electron Microscopy Sciences; Hatfield, PA).

2.1.2 Confocal imaging of FluoSpheres—A Leica TCS SPE confocal microscope (Leica, Mannheim, Germany) was used to acquire z-stacks through FluoSpheres using a 63x oil immersion objective with a 5.61 zoom factor. Lateral and axial sampling parameters were 61 nm and 130 nm, respectively, and were identical to those used to image our dendritic segments. All z-stacks were maintained at a xy pixel distribution of 512×512 and scanned at a frequency of 400 Hz. FluoSpheres were imaged using either Type FF immersion oil (refractive index: 1.47; Cargille, Cedar Grove, NJ) or Type LDF immersion oil (refractive index: 1.515; Cargille, Cedar Grove, NJ).

2.1.3 Point spread function analysis—FluoSphere z-stacks were opened in FIJI (National Institute of Health; Bethesda, MD) where a single FluoSphere was cropped and analyzed using the MetroloJ plug-in. MetroloJ generated a PSF report detailing the full width and half maximum (FWHM), the standard measurements for fluorescent objects, in the lateral and axial directions. PSF reports were generated for 4-5 FluoSpheres in each condition and the average was reported for FWHM in Figure 1.

2.2 Analyses of Dendritic Spine Densities

2.2.1 Animals—Female Sprague Dawley rats 12 weeks of age (175-200g) from Harlan labs (Indianapolis, IN) were housed in pairs, handled daily, and allowed to habituate to the laboratory for one week prior to experimentation. Animals were maintained on a 12 hr light-dark cycle with lights on at 6:00 am. All animal procedures were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee at the University of Minnesota.

The animals were anesthetized using Beuthanasia-D (0.3 ml i.p./animal, Schering, Union, NJ), injected with 0.25 ml heparin into the left ventricle of the heart and intracardially perfused with 25 mM phosphate buffered saline (PBS, pH = 7.2) for 3 min at 25 ml/min followed by ice-cold 1.5% paraformaldehyde in 25 mM PBS for 20 min. Perfusion with 1.5% paraformaldehyde results in more complete and clearer visualization of DiI-labeled neurons when compared to perfusion with the standard 4% paraformaldehyde (Staffend and Meisel 2011b). Brains were removed and coronally blocked to allow for penetration of the

post-perfusion fixative (1.5% paraformaldehyde for 1 hr) into the tissue blocks containing the striatum. Brains were sectioned at 300 μm in serial, coronal sections through the striatum using a Vibratome (Lancer Series 1000, St. Louis, MO). Sections were placed in wells containing 25 mM PBS until DiI labeling.

2.2.2 DiI labeling—Instructions for DiI labeling are described in detail by Staffend and Meisel (2011a). Briefly, microcarriers containing DiI-coated tungsten particles (2 mg lipophilic carbocyanine DiI (Molecular Probes, Carlsbad, CA) dissolved in 100 μl of dichloromethane mixed with 90 mg of 1.3 μm tungsten particles (Biorad, Hercules, CA)) were made in Tefzel tubing (Biorad) pre-coated with freshly prepared 10-15 mg/ml polyvinylpyrrolidone (PVP, Sigma-Aldrich, St. Louis, MO) and cut into 1.3 mm segments. A Helios Gene Gun (BioRad) with a modified barrel, 40 mm spacer and 70 μm nylon mesh filter was used to deliver the microcarriers to the lightly fixed brain sections using helium gas at a pressure of 100 PSI. Prior to DiI delivery, PBS was removed from wells containing the brain sections. Immediately following DiI delivery, brain sections were re-submerged in PBS for 24 hrs in the dark at room temperature for diffusion of DiI. After 24 hrs, the PBS was removed and replaced with 4% paraformaldehyde in PBS for 1 hr at room temperature. Sections were washed in PBS then mounted on Superfrost slides and coverslipped with 5% n-propyl-gallate (w/v) in glycerin or Fluorglo mounting media for lipophilic dyes.

2.2.3 Confocal imaging—A Leica TCS SPE confocal microscope (Leica, Mannheim, Germany) was used to acquire z-stacks of DiI labeled whole neurons and dendritic segments. Whole neurons were imaged using a Leica PLAN APO 20X, 0.7 NA air objective (11506166; Leica, Mannheim, Germany), a pixel distribution of 512×512 , at a frequency of 400 Hz, with pinhole size set to 1 Airy. Each neuron was scanned at 1.0 μm increments in the z-axis. Leica LAS AF software was used to reconstruct z-stacks of whole neurons in order to trace the actual length of a dendrite from the soma. Target dendritic segments 70-200 μm from the soma in medium spiny neurons of the striatum were imaged with a Leica PLAN APO 63X, 1.4 NA oil immersion objective (11506187; Leica, Mannheim, Germany), a pixel distribution of 512×512 , at a frequency of 400 Hz, with pinhole size set to 1 Airy and a zoom of 5.61. Z-stacks of dendritic segments were acquired with a lateral sampling of 61 nm at 0.13 μm increments in the z-axis. By adjusting the laser power and photomultiplier, each dendrite was imaged in its full dynamic range where the saturation threshold was achieved at dendritic spine heads. Separate dendritic segments were imaged using either Type FF immersion oil (refractive index: 1.47; Cargille, Cedar Grove, NJ) or Type LDF immersion oil (refractive index: 1.515; Cargille, Cedar Grove, NJ). For each brain region, 2 to 3 neurons and 3 dendritic segments per neuron were imaged to generate a total of 6 to 9 segments for each brain region from every animal. Depending on the experiment and treatment conditions, a final sample size of 3-8 animals was obtained for each brain region per treatment group (see figure legends for group sample sizes).

2.2.4 Quantitation—Confocal z-stacks of dendritic segments were first subjected to a 3D-deconvolution process using Autoquant X AutoDeblur Gold CF software (Media Cybernetics, Bethesda, MD; version 2.2 to evaluate the effect of mounting medium on dendritic spines and version 3.0 to evaluate the effect of immersion oil on dendritic spines).

Deconvoluted images were then reconstructed in 3D using the Imaris software (Bitplane Inc., St. Paul, MN; version 7.1 in experiment 1 and version 7.6 in experiment 2), with dendritic shaft and spines traced manually in the xy plane using the Filament tool and the Autodepth function to generate dendritic spine density normalized to 10 μm of dendritic length. A contrast threshold of 0.4-0.7 enabled accurate 3D reconstruction of dendritic shaft and spines.

2.2.5 Data analysis—For each animal, 2-3 neurons were analyzed in each brain region, though each animal was used as the unit of statistical analysis. Independent sample t-tests were used to evaluate statistical the effects of mounting medium and immersion oil for each brain region using Graph Pad Prism 5 software (La Jolla, CA). For all statistical tests, results were considered to be statistically significant if $p < 0.05$.

3. Results

3.1 Quantitative and qualitative analysis of point spread functions from FluoSpheres mounted with two different mediums and imaged with two different immersion oils

Initially, we took a materials approach using fluorescent beads to evaluate how mounting media and immersion oils influenced imaging of a point source of light. The 3D distortion pattern, i.e., point spread function, generated by confocal microscopy determines the resolution limit of an optical system (Pawley 2010). The point spread function can be measured by imaging an object smaller than the resolution limit, such as a 100 nm fluorescent microspheres (FluoSphere) used in our study. To determine the point spread function generated by our imaging parameters of dendritic segments, we followed previously published protocols (Cole et al. 2011). First, we prepared FluoSphere samples mounted in either glycerin-based mounting medium (5%-npropylgallate in glycerin) or Fluorglo mounting medium. Next, we imaged the samples under dendritic segment imaging parameters using either Type FF or Type LDF immersion oil. Figure 1 shows qualitative and quantitative measures of point spread function in lateral and axial planes for glycerin-based mounting medium with Type FF immersion oil (left), Fluorglo mounting medium with Type FF immersion oil (middle) and Fluorglo mounting medium with Type LDF immersion oil (right). Quantitative measures of point spread function from these images were reported in the standard measurement for size of object in fluorescent images, full width at half maximum (FWHM). In the first analysis, the lateral and axial FWHM of FluoSpheres imaged with Type FF immersion oil was found to be numerically higher in the glycerin-based mounting medium compared to the Fluorglo mounting medium. In the second analysis, FluoSpheres mounted in Fluorglo had lower lateral and axial FWHM values when imaged with Type LDF immersion oil compared with FluoSpheres imaged with Type FF immersion oil.

3.2 Qualitative point spread function analyses of dendritic segments from striatal medium spiny neurons mounted with two different mediums and imaged with two different immersion oils

Next, we sought to determine whether the effects of mounting medium and immersion oil that we observed from imaging FluoSpheres were applicable to imaging dendritic segments.

Figure 2 shows quantitative measures of point spread function in lateral and axial planes for the same three conditions outlined in Figure 1. Consistent with the data obtained from the *FluoSpheres*, changing the mounting media from 5%-npropylgallate in glycerin to Fluorglo reduced the light scatter in the z-plane. Furthermore, use of the Type LDF immersion oil further improved the quality of the axial image.

3.3 Mounting Medium under some conditions affects the ability to accurately quantify spine densities

While the information obtained from both the *FluoSpheres* and the axial images of dendritic spines indicate improvement of the 3D reconstruction using Fluorglo mounting medium, we wanted to empirically test whether this would translate into an increased ability to resolve dendritic spines. To do so, we compared dendritic spine densities of medium spiny neurons (MSNs) imaged from striatal sections that were mounted in either 5%-n-propyl gallate in glycerin or in Fluorglo medium. We found that the Fluorglo media increased the ability to visualize dendritic spines from MSNs of the caudate-putamen (CPu) (Figure 3; independent sample t-test; $t(13) = 2.28, p < 0.05$). In contrast, altering the mounting media did not significantly affect the quantification analyses of the nucleus accumbens core (NAcC) and nucleus accumbens shell (NAcSh) (see Discussion).

3.4 Immersion oil has a consistent effect on dendritic spine densities obtained from medium spiny neurons in three striatal regions

In the last experiment, we determined whether mounting medium affects the ability to resolve individual dendritic spines within these three brain regions. Dendritic spine densities from striatal and nucleus accumbens sections were mounted with Fluorglo medium and imaged using either Type FF or Type LDF immersion oil. The ability to resolve individual dendritic spines in all three brain regions was affected by the immersion oil. Specifically, LDF immersion oil increased the ability to visualize individual spines (Fig. 4, independent sample t-test; CPu: $t(6) = 2.52, p < 0.05$; NAcC: $t(6) = 3.76, p < 0.05$; NAcSh: $t(6) = 4.27, p < 0.05$).

4. Discussion

The resolution of 3D images obtained from confocal microscopy not only depends on the ability to detect a light source, but also the ability to resolve different sources of light. Thus, selection of mounting media and immersion oil would seem to be of great importance, particularly when imaging small, spatially constricted structures such as dendritic spines. Thus, while the appropriate optimization of these variables may seem apparent, these variables are often overlooked (and rarely reported) when quantitative analyses of dendritic spines are published. Alternatively, with a lack of quantitative data to validate the impact of mounting media and immersion oil on the ability to resolve distinct biological structures, altering these variables could be considered to reflect theoretical limitations of an imaging system, and not germane from a practical standpoint. Hence, our goal was to understand the effect of these two variables using confocal microscopy in a biologically relevant context.

First, we compared two frequently utilized mounting medias. In biological preparations, glycerin-based medias are quite adaptable – allowing various optics and chromophores to be utilized. This was compared to Fluorglo, an example of a fluorescent-compatible hardening compound. The hardening of the media helps limit movement of the sample when imaging, but this compound is less versatile than glycerin-based medias. Data from both the FluoSpheres and dendritic spines indicate Fluorglo slightly improved optic performance. In one brain region (i.e. caudate putamen) this translated into an increased ability to resolve dendritic spines, whereas in both the core and shell regions of the nucleus accumbens, our quantitative analyses were unaffected. Our impression is that the improved resolution with Fluorglo may be a function of the hardening properties of this mounting medium, as media that hardens as it dries minimizes the distortion of the z-plane caused by the weight of the coverglass and pressure of the objective. Alternatively, the refractive index of the glycerin-based mounting media is 1.47. This compares to Fluorglo, with a refractive index of 1.42. This difference may also contribute to the disparity in resolution. Nevertheless, it would seem use of a hardening compound would be the preferred mounting media in these types of studies, although glycerin-based medias offer a reasonable alternative if required.

In contrast, selection of the appropriate immersion oil seems essential. There has been the question regarding whether the refractive index of the immersion oil should match the tissue and mounting media, or the glass through which the sample is imaged. Both Type FF and Type LDF are commonly used immersion oils for these reasons. Specifically, in our setup, the refractive index of the Type FF immersion oil (RI: 1.47) matched that of the tissue and mounting medium, whereas the refractive index of the Type LDF (RI: 1.51) immersion oil matched that of the glass. From our quantitative data, there is marked improvement of the optical image when using an immersion oil (Type LDF) matching the glass through which the sample is visualized. In all three striatal subregions, dendritic spine densities from sections imaged with Type LDF immersion oil were greater than those imaged with Type FF immersion oil. Differences in the point spread functions in the lateral plane and more dramatically in the axial plane of the FluoSpheres imaged with Type LDF immersion oil support our measurements in the dendritic segments. Thus we attribute the increased spine density observed with Type LDF immersion oil to an increase in ability to resolve individual spines especially in the axial plane. Our data emphasize that to obtain the best optical resolution, the refractive index of immersion oil should match that of the glass through which the sample is visualized rather than the refractive index of the medium that a sample is mounted within. Consequently, selecting immersion oil based on refractive index of the microscopic objective is crucial in reliably resolving individual dendritic spines.

Our data highlight the importance of mounting medium and immersion oil selection in confocal imaging. Yet, the choice of mounting medium or immersion oil are commonly omitted in experimental methods. This is key because differences in dendritic spine density values across laboratories are often greater than a significant treatment outcome within a single laboratory. Dumitru and colleagues (2012) noted that divergent data trends have been reported depending on whether dendritic spine analyses were conducted in 2D or 3D. Taken together with our results demonstrating that mounting medium and immersion oil can affect the ability to resolve individual spines in both the lateral and axial planes, immersion oil is a crucial variable, especially for 3D confocal analysis. As changes in dendritic spine density

reflect changes in neuroanatomical circuitry, the inconsistency in data trends originating from imaging and analytical methods have lead to ambiguous conclusions regarding relationships among synaptic connectivity, neuronal function and behavior. Generating more accurate 3D representations with confocal imaging can improve the ability clarify these neural structure/function relationships.

Acknowledgements

This material is based upon work supported by the National Institutes of Health grant DA035008 (PGM and RLM), the National Institute of Health grant DA013680 (RLM), and a National Science Foundation Grant No. 00006595 (BMP). We would like to thank Dr. Martin Wessendorf and the University of Minnesota University Imaging Center, particularly Guillermo Marques for advice and technical assistance.

References

- Alvarez VA, Sabatini BL. Anatomical and Physiological Plasticity of Dendritic Spines. *Annu Rev Neurosci.* 2007; 30(1):79–97. [PubMed: 17280523]
- Bhatt DH, Zhang S, Gan W-B. Dendritic Spine Dynamics. *Annu Rev Physiol.* 2009; 71(1):261–82. [PubMed: 19575680]
- Cole RW, Jinadasa T, Brown CM. Measuring and interpreting point spread functions to determine confocal microscope resolution and ensure quality control. *Nat Protoc.* 2011; 6(12):1929–41. [PubMed: 22082987]
- Dumitriu D, Laplant Q, Grossman YS, Dias C, Janssen WG, Russo SJ, et al. Subregional, dendritic compartment, and spine subtype specificity in cocaine regulation of dendritic spines in the nucleus accumbens. *J Neurosci.* 2012; 32(20):6957–66. [PubMed: 22593064]
- Dumitriu D, Rodriguez A, Morrison JH. High throughput, detailed, cell-specific neuroanatomy of dendritic spines using microinjection and confocal microscopy. *Nat Protoc.* 2011; 6(9):1391–411. [PubMed: 21886104]
- Pawley J. *Handbook of Biological Confocal Microscopy.* Springer Science & Business Media. 2010
- Staffend NA, Meisel RL. DiOlistic labeling in fixed brain slices: phenotype, morphology and dendritic spines. *Curr Protoc Neurosci.* 2011a Unit 2.13.
- Staffend NA, Meisel RL. DiOlistic Labeling of Neurons in Tissue Slices: A Qualitative and Quantitative Analysis of Methodological Variations. *Front Neuroanat.* 2011b; 5(1)

Highlights

- Mounting medium and immersion oil affect resolution in confocal microscopy
- Optimizing confocal resolution can impact biological measurements
- Select immersion oil to match refractive index of glass
- Mounting media may be selected based on tissue requirements

Fig. 1.

Mounting medium has an effect on axial, but not lateral resolution of imaged FluoSpheres, while immersion oil affects both lateral and axial resolution of imaged FluoSpheres. Whether FluoSpheres imaged with Type FF immersion oil, were mounted with glycerin or Fluorglo had no effect on lateral FWHM. Contrastingly, FluoSpheres imaged with Type FF immersion oil exhibited a slight decrease in axial FWHM when mounted with Fluorglo compared with glycerin. FluoSpheres mounted with Fluorglo that were imaged with the LDF immersion oil showed a small decrease in lateral FWHM and a large decrease in axial FWHM compared to FluoSpheres imaged with Type FF immersion oil. The full width at half maximum (FWHM) or standard measurement for fluorescent object value is the average obtained from 4-5 individual FluoSpheres. Scale bar is 1 μm .

Fig. 2.

Mounting medium and immersion oil each have a small effect on lateral resolution and a large effect on axial resolution of imaged striatal medium spiny neuron dendritic segments. When dendritic segments were imaged with Type FF immersion oil, sections mounted with Fluorglo medium showed a slight improvement in lateral resolution and a more dramatic improvement axial resolution compared to sections mounted with glycerin. Similarly, when sections were mounted with Fluorglo, dendritic segments imaged with Type LDF immersion oil showed a slight improvement in lateral resolution and a more dramatic improvement axial resolution compared to dendritic segments imaged with Type FF immersion oil. Scale bar is 50 μm .

Fig. 3.

Mounting medium affects striatal dendritic spine densities obtained from DiI-labeled medium spiny neurons in the caudate putamen (CPu), but not the nucleus accumbens (NAcC) or nucleus accumbens shell (NAcSh). **a** Compared with sections mounted with glycerin-based medium, sections mounted with Fluorglo mounting medium showed increased dendritic spine density of medium spiny neurons in the CPu. **b** Dendritic spine densities of medium spiny neurons of the NAcC were unaffected by mounting medium selection. **c** Dendritic spine densities of medium spiny neurons of the NAcSh were unaffected by mounting medium selection. The number inside of each bar represents the number of animals per treatment condition. Grey bars are significantly different than white bars $p < 0.05$

Fig. 4.

Immersion oil influences striatal dendritic spine densities obtained from DiI-labeled medium spiny neurons in the caudate putament (CPu), nucleus accumbens (NAcC) and nucleus accumbens shell (NAcSh). **a** Compared with dendritic segments of medium spiny neurons imaged with Type FF immersion oil, dendritic segments medium spiny neurons imaged with Type LDF immersion oil showed increased dendritic spine density in the CPu. **b** Compared with dendritic segments of medium spiny neurons imaged with Type FF immersion oil, dendritic segments medium spiny neurons imaged with Type LDF immersion oil showed increased dendritic spine density in the NAcC. **c** Compared with dendritic segments of medium spiny neurons imaged with Type FF immersion oil, dendritic segments medium spiny neurons imaged with Type LDF immersion oil showed increased dendritic spine density in the NAcSh. The number inside of each bar represents the number of animals per treatment condition. Grey bars are significantly different than white bars $p < 0.05$